THE SPECIFICITY OF THE ABDERHALDEN REACTION WITH VEGETABLE PROTEINS*

THE BIOLOGIC REACTIONS OF THE VEGETABLE PROTEINS, VIII

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It is now generally assumed that when a protein is introduced parenterally into the animal organism, antibodies are elaborated within the latter which lead to the destruction of that protein. Vaughan[1] believes this reaction to be universal enough to be incorporated into a biologic law. The nature of these antibodies, the seat of their formation, and the mode of their action are as yet unanswered questions. They are thought, however, to be able to cause cleavage of the protein molecule introduced, and the products of this cleavage may cause certain phenomena, which can be detected by the characteristic behavior of the animal employed. Anaphylaxis is an example of such phenomena. As early as 1910 Abderhalden attempted to establish the nature of the proteolytic ferments concerned in these reactions—protective ferments he called them. After adding some of the immunized animal's serum to the specific antigen in vitro, he noted as the result of protein digestion the appearance of dialyzable products, which he detected by various methods. He stated, moreover, that these ferments were specific and represented one of the responses of the animal body to the protein introduced. From these observations Abderhalden developed his reaction for the diagnosis of pregnancy. Abderhalden assumed that the complex cells of the chorionic villi enter the maternal blood stream during pregnancy and are there rendered innocuous by specific enzymes especially generated for that purpose. The presence of these so-called specific or protective ferments, just as in animal experimentation, could be demonstrated in vitro by adding a definite amount of the patient's serum to some of the specific protein assumed to be acting as antigen in this case, placenta. Specificity was likewise considered as demonstrated by the detection, by the same methods, of dialyzable products resulting from the interaction of serum and sub-

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[1] Protein Split Products in Relation to Immunity and Disease, 1913, p. 25.

strate. The serum was thought to be unable to interact with any other substrate than placental protein. Apparently, then, these ferments develop whether the protein enters the blood stream parenterally from within, or from without, the animal body. In either case they were held by him to be specific and to be demonstrable by the same methods.

Abderhalden's application of his theory of protective ferments and his methods for detecting the presence of the latter have stimulated a vast amount of work, and consequently a very extensive literature has developed. Since there are at present several elaborate reviews of this literature, it is deemed unnecessary to add another here. It is probably sufficient to state that there is no concordance of opinion among investigators as to the mechanism or the specificity or nonspecificity of the reaction.

The bulk of experimental work has been done with animal materials obviously more or less complex as to nature and composition. The use of especially prepared pure isolated proteins instead, such, for example, as are obtained from vegetable sources, seemed highly desirable, since it would tend to eliminate many serious and noteworthy objections. If the animal body responds to the parenteral introduction of a mixture of proteins by the generation of ferments more or less specific, it should be able to do so all the more specifically on the introduction of a single pure and extremely foreign protein. With the intent, then, of removing some of the uncertainty now existing as to the specificity of the Abderhalden reaction, this work was undertaken using as antigen carefully purified preparations of vegetable proteins obtained from Dr. T. B. Osborne. These proteins, furthermore, offer the added advantage of having been extensively studied by other immunologic methods, by Osborne, Wells, Lake, and White.[2]

The methods employed in making the preparations of the vegetable proteins used in this investigation and the chief chemical characteristics of these substances have been described by Osborne.[3] The immunologic reactions produced by them have also been discussed by Wells and Osborne in earlier papers of this series. Crude extracts of plant seeds have been employed with success for similar purposes by earlier workers. Both the isolated vegetable proteins and the plant extracts have also been used to some extent in immunizing animals for the Abderhalden test. Abderhalden, injecting gliadin, edestin, and casein,

[2] Jour. Infect. Dis., 1911, 8, p. 66. This paper also contains an historical review of the use of plant extracts in immunologic reactions. Ibid., 1913, 12, p. 341; 13, p. 103; 1914, 14, pp. 364, 377; 1915, 17, p. 259; 1916, 19, p. 183.

[3] Ergebn. d. Physiol., 1910, 10, p. 47.

and peptone from gelatin, edestin, and casein, obtained ferments which he maintained are specific for those substances. Issatschenko obtained similar results, using as antigen extracts of hempseed, wheat flour, hazelnut, and oats. He found that the serum decomposed the proteins in the extracts used for injection, but not the others. However, the immune serum would not differentiate between extracts from two closely allied plants, such as two species of flax, when the one was employed as antigen. In this sense the serum was not specific. Denying entirely the specificity of the Abderhalden reaction, Herzfeld[4] found that the sera of all normal, pregnant, and neurotic persons investigated by him were capable of digesting zein, the alcohol-soluble protein from corn, as well as the albumins from egg or blood, fibrin from blood, and casein from cow's milk. Nitzescu[5] concluded on the basis of his work that persons suffering from pellagra have a ferment in their blood specific for zein. He found that the sera of all patients for whom a diagnosis of pellagra was certain, gave a positive Abderhalden reaction with zein. Twelve normal peasants on corn diet gave a negative reaction. In the hospital, of 14 patients with pellagra receiving bread, 10 gave negative reactions with gliadin from wheat and 4 indefinite reactions.

In addition to their proved antigenic properties, the vegetable proteins are highly satisfactory in work with the Abderhalden reaction because they do not tend to dialyze through animal membranes and in themselves give but a slight color reaction when boiled directly with ninhydrin. Moreover, when results discordant with specificity are obtained, the objection cannot be raised that the discrepancy is ascribable to blood cells or dialyzable substances remaining in the substrate. This latter feature suggested to von L. Flatow[6] the advantage of using casein in a similar study. He further emphasizes the fact that utilizing such pure materials as casein tends to eliminate the presence of proteins common to nearly all tissues, such, for example, as are present in connective tissue, whereby specificity might be clouded. Again, vegetable protein from the same sample can be employed repeatedly as substrate, this advantage enhancing the value of comparative results over those obtained by using samples from various organs—such as placenta— which have undergone autolytic digestion to different degrees. Bacterial infection is also less imminent in the

[4] Deutsch. med. Wchnschr., 1915, 41, p. 1151.
[5] Ibid., 1914, 40, p. 1614.
[6] München. med. Wchnschr., 1914, 61, 468.

vegetable proteins, which can be kept in dry condition in bottles, than in placental or other animal tissue preserved under toluene. In a word, these vegetable proteins in many cases represent as pure antigens as can be isolated, while most of the animal materials that have been studied represent mixtures of antigens.

EXPERIMENTAL WORK

Large vigorous male rabbits were almost exclusively employed. They were injected with the specific protein (usually intraperitoneally) in amounts and over a period of time as indicated in the tables. The intravenous method of immunization was not employed as being fraught with too much danger to the animals. Care had to be exercised not to inject too large a dose of the protein at one time, as fatal toxic action might result; closely repeated doses, given every day or every second day, of small amounts, varying from 0.1 to 0.2 gm., dissolved in a 0.1% NaOH solution were more efficient. Blood was obtained usually from the ear of the animal, sometimes directly from the heart.

The preparation of the serum and the method of dialysis as outlined by Abderhalden in the fourth edition of his "Abwehrfermente" were followed closely. Slight modifications, which would not depreciate the comparative value of the results, were introduced from time to time as seemed necessary to meet existing conditions. The serum was usually, but not invariably, free from hemoglobin. It was not examined spectroscopically. Hemoglobin-tinted serum was usually tested, however, as to its reactive power exactly as was the clear serum, for it was observed by us as well as by other workers that hemoglobin in the serum does not necessarily vitiate its usefulness. (Abderhalden insists that the sera must be absolutely free from hemoglobin even to spectroscope examinations.) Moreover, it is not always possible to obtain hemoglobin-free rabbit serum, even if the utmost precautions are exercised.

A very grievous difficulty exists in the use of rabbit serum in that it frequently contains dialyzable substances in amounts sufficient in themselves to give a strong positive reaction with ninhydrin. To eliminate this source of error part of the serum employed was predialyzed to running salt solution (0.85%) for periods ranging from 6 to 8 hours, before it was added to the substrates. This procedure usually served to remove all dialyzable substances and has been recommended by some investigators for use in all sera. With the object of reducing the amount of these dialyzable substances as much as possible, the rabbits were kept without food from 10 to 24 hours (a few, 36 hours) before drawing the blood.

The diffusion tubes of Schleicher and Shüll, No. 579a, were employed exclusively in the dialysis. They were frequently tested as outlined by Abderhalden. Each tube was numbered to correspond to a definite protein and was retained for that protein as exclusively as possible. Dialyzing flasks prepared for the Abderhalden reaction were found to be very serviceable as containers for the diffusion tubes.

In performing an experiment ordinarily 1 c.c. of serum was added to 0.1 gm. of the protein. Some variations were made in these amounts as are indicated later. Digestion continued for periods of time ranging from 16 to 24 hours, at a temperature of approximately 37 C., tho unavoidably some tests ran for a slightly longer period. The ninhydrin test was then made according to the

method laid down by Abderhalden. All experimental conditions were maintained as nearly uniform as possible at all times—this being deemed necessary to produce results of any comparative value. Falls[7] in a recent article especially emphasizes this feature. The depth of color obtained on boiling the dialyzed and ninhydrin solutions together is indicated by the symbols conventionally employed in immune reactions for that purpose; thus "+ + +" for a very deep blue-purple color, "+ +" for a less deep color, and so on, read after 30 minutes' cooling. When the dialysate from the serum alone gave some reaction, the experiment was counted as positive only when the difference in depth of color between the dialysate of the serum, and that of the protein and serum was marked enough to merit such a decision beyond any possible doubt. If the two dialysates showed a reaction of about the same degree the experiment was considered questionable. This procedure has support in the work of Pearce and Williams,[8] and in the directions of Abderhalden. There is no apparent reason why the view is not tenable.

The sera of many of the rabbits were tested for the presence of reacting substances against the individual proteins, before any immunization had taken place. At no time did a reaction develop after the period of incubation designated, which could not be ascribed to dialyzable substances present in the normal serum itself: that is, there was no apparent interaction between the normal serum and protein substrate. Because of these negative findings, it was not deemed necessary to subject the sera of all the rabbits to this test. After immunization it was not always possible to procure sufficient serum at any given time to run the full quota of tests; hence some irregularities may appear in the number of reactions obtained from the various proteins. No attempt was made at this stage of the work to determine how quickly antibodies to these various antigens appeared in the animals' sera, which could be detected by the dialysis method, nor to determine what the nature of these antibodies is. The serum was usually obtained from 3 to 4 days subsequent to the last previous injection of protein.

The proteins used as antigens were (A) edestin, a globulin from the hempseed (Cannabis sativa); (B) a globulin from the squash seed (Cucurbita maxima); (C) gliadin, an alcohol-soluble protein from wheat (Triticum vulgare); (D) hordein, an alcohol-soluble protein from barley (Hordeum vulgare); (E) pea legumin, a globulin from the pea (Pisum sativum); (F) phaseolin, a globulin from the adzuki bean (Phaseolus radiatus); (G) glycinin, a globulin from the soy bean (Sojahi spida); (H) fresh egg white. The nature and results of experiments are herewith recorded.

A. With Edestin as Antigen

Rabbit 1.—Injected with a total of 4.5 gm. of edestin from March 8 to June 26, 1915. Blood was drawn and serum obtained April 12, 21, 30, May 12, 24, 1915. The experiments of April 12, May 12, and May 24 were questionable and are discarded because of the presence of sufficient dialyzable substances in the serum to obscure the definiteness of the ninhydrin reaction in differentiating between the serum and protein, and the serum alone. In this series as in the subsequent ones, the Arabic figures designate the number of a given experiment for a particular rabbit, while the Roman numerals indicate the position of the same experiment in the series for the given protein antigen.

[7] Jour. Am. Med. Assn., 1914, 63, p. 1172.

[8] Jour. Infect. Dis., 1914, 14, p. 351.

TABLE 1

RESULTS OF ABDERHALDEN TEST EMPLOYING EDESTIN AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, I	April 21	22	0.01 edestin	1	+
				1*	Trace
2, II	April 30	22	0.01 edestin	1	++
			0.01 gliadin (wheat)	1	Strong trace
				1*	Strong trace

* Control.

Rabbit 2.—Injected with a total of 4.2 gm. of edestin from April 12 to May 28, 1915. Blood was drawn and serum obtained April 17, 26, 29, May 20. and 29. The experiments of April 17 and May 29 were questionable and are discarded because of the presence of sufficient dialyzable substances in the serum to obscure the definiteness of the ninhydrin reaction in differentiating between the serum and protein, and the serum alone.

TABLE 2

RESULTS OF ABDERHALDEN TEST EMPLOYING EDESTIN AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, III	April 27	23	0.01 edestin	1	++
				1*	Faint trace
2, IV	April 29	24	0.01 edestin	1	++
				1*	Faint trace
			0.01 gliadin (wheat)	0.75	Faint trace
				0.75*	Faint trace2
3, V	May 1	23	0.01 edestin	1	+ Strong
			0.01 globulin (squash seed)	1	0
			0.01 hordein	1	0
			0.01 legumin (pea)	1	0
			0.01 legumin (horse bean)	1	0
			0.01 globulin (black walnut)	1*	0

* Control.

Rabbit 3.—Injected with a total of 2 gm. of edestin from Oct. 15, to Oct. 27, 1915. Blood was drawn and serum obtained Oct. 26. Only one experiment was performed on this animal, as it died from the toxic effects of an over dose of the protein on Oct. 28. Blood was drawn immediately after death from the heart. This result is presented with some hesitancy on account of the extreme hemoglobin content of the sera, probably due to the fact that the blood had stood in the icebox over night.

TABLE 3

RESULTS OF ABDERHALDEN TEST EMPLOYING EDESTIN AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, VI	Oct. 27	23	0.01 edestin	1	++
			0.01 excelsin	1	0
				1*	0

* Control.

Rabbit 4.—Injected with a total of 3.1 gm. of edestin from Nov. 1 to Dec. 6, 1915. Blood was drawn and serum obtained Nov. 29 and Dec. 7. The experiment of Dec. 7 is discarded as the rabbit at that time appeared ill from "snuffles."

TABLE 4

RESULTS OF ABDERHALDEN TEST EMPLOYING EDESTIN AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, VII	Nov. 29	19	0.1 edestin	1	Trace
			0.1 globulin (squash seed)	1	Trace
			0.1 excelsin	1	+ (?)
			0.1 gliadin (wheat)	1	++
			0.1 legumin (pea)	1	Trace
				1*	Trace

* Control.

Rabbit 5.—Injected with a total of 1.7 gm. of edestin from Jan. 17 to March 13, 1916. Blood was drawn and serum obtained Feb. 11 and 22, and March 3. The serum of March 3 was predialyzed to running salt solution (0.85%) for 8½ hours.

TABLE 5

RESULTS OF ABDERHALDEN TEST EMPLOYING EDESTIN AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, VIII	Feb. 11	20	0.1 edestin	1	++
			0.05 edestin	1.5	++ Strong
				1.5*	Trace
			0.1 globulin (squash seed)	1	Trace
			0.1 excelsin	1	+
			0.1 gliadin (wheat)	1	Strong trace
			0.1 gliadin (rye)	1	Trace
			0.1 legumin (pea)	1	+
			0.1 hordein	1	0
			0.1 egg white, dry	1	0
				1*	Trace
2, IX	Feb. 22	18	0.1 edestin	1	+ Strong
			0.2 edestin	1.5	+ Strong
				1.5*	Trace
			0.1 globulin (squash seed)	1	0
			0.05 excelsin	1	0
			0.1 gliadin (wheat)	1	+
			0.1 legumin (pea)	1	+
			0.1 hordein	1	0
			0.1 egg white, dry	1	0
				1*	0
3, X	Mar. 3	18	0.1 edestin	1.5	+
				1.5*	0
			0.2 edestin	1	Trace ?
			0.1 globulin (squash seed)	1	0
			0.1 gliadin (wheat)	1	0
			0.1 legumin (pea)	1	0
			0.1 hordein	1	0
			0.1 egg white, dry	1	0
				1*	0

* Control.

Rabbit 6.—Injected with a total of 1.7 gm. of edestin from Jan. 17 to March 13, 1916. Blood was drawn and serum obtained Feb. 11 and 22, and March 3, 1916. The sera of Feb. 11 and 22 were predialyzed to running salt solution (0.85%) for 7 hours.

TABLE 6

RESULTS OF ABDERHALDEN TEST EMPLOYING EDESTIN AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, XI	Feb. 11	23	0.1 edestin	1	Strong trace
			0.2 edestin	1.5	+
			0.05 edestin	1.5	0
				1.5*	0
			0.1 globulin (squash seed)	1	+
			0.1 excelsin	1	0
			0.1 gliadin (wheat)	1	Strong trace
			0.1 gliadin (rye)	1	+
			0.1 legumin (pea)	1	Trace ?
			0.1 hordein	1	+
			0.1 egg white, dry	1	0
				1*	0
2, XII	Feb. 22	17	0.1 edestin	1	++
			0.2 edestin	1.5	++
				1.5*	0
			0.1 globulin (squash seed)	1	0
			0.05 excelsin	1	0
			0.1 gliadin (wheat)	1	0
			0.1 gliadin (rye)	1	+
			0.1 legumin (pea)	1	0
			0.1 hordein	1	0
			0.1 prolamin (oat)	1	0
			0.1 egg white, dry	1	0
				1*	0
2, XIII	Mar. 3	18	0.1 edestin	1.5	0
				1.5*	0
			0.2 edestin	1	++
			0.1 globulin (squash seed)	1	0
			0.05 excelsin	1	+
			0.1 gliadin (wheat)	1	Trace
			0.1 legumin (pea)	1	0
			0.1 hordein	1	0
			0.1 egg white, dry	1	0
			0.1 prolamin (oat)	1	+
			0.1 globulin (castor bean)	1	0
			0.1 globulin (cocoanut)	1	+
			0.1 alcohol-sol. protein from millet	1	0
				1*	0

* Control.

A summary of the experiments with edestin is given in Table 7.

TABLE 7

SUMMARY OF THE RESULTS OF ABDERHALDEN TESTS EMPLOYING EDESTIN AS ANTIGEN

Protein	Number of Tests	"++ Strong" or "++"	"+ Strong" or "+"	Strong Trace	Trace or Faint Trace	Questionable	No Reactions
Edestin	18	9	6	1	0	1	1
Globulin (squash seed)	6	0	1	0	0	0	5
Excelsin	7	0	2	0	0	1	4
Gliadin (wheat)	7	1	1	2	1	0	2
Gliadin (rye)	2	0	2	0	0	0	0
Legumin (pea)	7	0	2	0	0	1	4
Hordein	7	0	1	0	0	0	6
Prolamin (oat)	2	0	1	0	0	0	1
Egg white, dry	6	0	0	0	0	0	6
Globulin (castor bean)	1	0	0	0	0	0	1
Cocoanut protein	1	0	1	0	0	0	0
Alcohol soluble protein from millet	1	0	0	0	0	0	1
Legumin (horse bean)	1	0	0	0	0	0	1
Globulin (black walnut)	1	0	0	0	0	0	1

B. WITH SQUASH-SEED GLOBULIN AS ANTIGEN

Rabbit 1.—Injected with a total of 5.8 gm. of squash-seed globulin from Oct. 16 to Dec. 10, 1915. Blood was drawn and serum obtained Nov. 2 and 27, and Dec. 10. The experiment of Nov. 2 was questionable, as the animal's blood apparently contained an excess of dialyzable substances, in spite of previous starvation. However, the differences in color reaction were sufficient to warrant its being included in the tabulations.

TABLE 8

RESULTS OF ABDERHALDEN TEST EMPLOYING SQUASH-SEED GLOBULIN AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, I	Nov. 2	23	0.01 globulin (squash seed)	1.5	+ Strong
			0.01 excelsin	1.5	Strong trace
				1.5*	Strong trace
2, II	Nov. 27	24	0.1 globulin (squash seed)	1	+
			0.1 excelsin	1	++
			0.1 gliadin (wheat)	1	Strong trace
			0.1 legumin (pea)	1	0
			0.1 edestin	1	0
				1*	0
3, III	Dec. 10	17	0.1 globulin (squash seed)	1	0
			0.1 globulin (squash seed)	1	0
			0.1 excelsin	1	0
			0.1 gliadin (wheat)	1	0
			0.1 gliadin (rye)	1	0
			0.1 legumin (pea)	1	+
			0.1 edestin	1	0
			0.1 hordein	1	Strong trace
			0.1 prolamin (oat)	0.75	Trace
				1*	Trace

* Control.

Rabbit 2.—Injected with a total of 7.8 gm. of squash-seed globulin from Oct. 22, 1915, to March 13, 1916. Blood was drawn and serum obtained Nov. 23, Dec. 10, 1915, Jan. 8, 21, Feb. 24, and March 7, 1916. The experiment of Jan. 8 is questionable on account of strong reactions obtained with serum alone, due apparently to an excess of dialyzable substances in the serum. The sera of Jan. 21 and Feb. 24 were predialyzed to running salt solution (0.85%) for 6 hours.

TABLE 9

RESULTS OF ABDERHALDEN TEST EMPLOYING SQUASH-SEED GLOBULIN AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, IV	Nov. 23	19	0.01 globulin (squash seed)	1	+
			0.01 excelsin	1	Strong trace
			0.01 edestin	1	0
				1*	0
2, V	Dec. 10	17	0.01 globulin (squash seed)	1	Strong trace
			0.1 excelsin	1	+
			0.1 gliadin (wheat)	1	+
			0.1 gliadin (rye)	1	0
			0.1 legumin (pea)	1	Trace
			0.1 edestin	1	0
			0.1 hordein	1	Strong trace
			0.1 fresh egg white (concentrated)	1	Trace
				1*	0
3, VI	Jan. 21	18	0.1 globulin (squash seed)	1	0
			0.1 excelsin	1	Strong trace
			0.1 gliadin (wheat)	1	+
			0.1 gliadin (rye)	1	Strong trace
			0.1 legumin (pea)	1	+
			0.1 edestin	1	0
			0.1 hordein	1	0
				1*	0
4, VII*	Jan. 21	18	0.1 globulin (squash seed)	1	0
			0.1 excelsin	1	0
			0.1 gliadin (wheat)	1	+
			0.1 legumin (pea)	1	0
			0.1 hordein	1	0
				1*	0
5, VIII	Feb. 24	17	0.1 globulin (squash seed)	1	0
			0.5 excelsin	1	0
			0.1 gliadin (wheat)	1	Strong trace
			0.1 gliadin (rye)	1	0
			0.1 legumin (pea)	1	Strong trace
			0.1 edestin	1	0
			0.1 hordein	1	0
			0.1 egg white, dry	1	0
				1	0
6, IX	Mar. 7	18	0.1 globulin (squash seed)	1	++
			0.2 globulin (squash seed)	1.5	0
				1.5*	0
			0.1 excelsin	1	0
			0.1 gliadin (wheat)	1	Trace
			0.1 legumin (pea)	1	++
			0.1 edestin	1	0
			0.1 hordein	1	0
				1*	0

* Control.

† The serum in Experiment VII is of the same sample as that in Experiment VI, but it was predialyzed for 6 hours before use.

Rabbit 3.—Injected with a total of 2.25 gm. squash-seed globulin from Jan. 12 to March 13, 1916. Blood was drawn and serum obtained Feb. 24 and March 7. The serum of March 7 was predialyzed to running salt solution (0.85%) for 7 hours.

TABLE 10

RESULTS OF ABDERHALDEN TEST EMPLOYING SQUASH-SEED GLOBULIN AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, X	Feb. 24	19	0.1 globulin (squash seed)	1	+
			0.2 globulin (squash seed)	1.5	++
				1.5*	0
			0.05 excelsin	1	0
			0.1 gliadin (wheat)	1	0
			0.1 gliadin (rye)	1	0
			0.1 legumin (pea)	1	0
			0.1 edestin	1	0
			0.1 hordein	1	0
			0.1 prolamin (oat)	1	0
			0.1 egg white, dry	1	0
			0.1 globulin (castor bean)	1	+
			0.1 cocoanut protein	1	0
				1*	0
2, XI	Mar. 7	18	0.1 globulin (squash seed)	1	Trace
			0.2 globulin (squash seed)	1	Trace
				1.5*	0
			0.1 excelsin	1	0
			0.1 gliadin (wheat)	1	Trace
			0.1 legumin (pea)	1	Trace
			0.1 edestin	1	0

* Control.

A summary of the experiments with squash-seed globulin is given in Table 11.

TABLE 11

RESULTS OF ABDERHALDEN TEST EMPLOYING SQUASH-SEED GLOBULIN AS ANTIGEN

Protein	Number of Tests	"++ Strong" or "++"	"+ Strong" or "+"	Strong Trace	Trace or Faint Trace	Questionable	No Reactions
Globulin (squash seed)	15	2	4	1	2	0	6
Excelsin	10	1	1	2	0	0	6
Gliadin (wheat)	9	0	3	2	2	0	2
Gliadin (rye)	5	0	0	1	0	0	4
Legumin (pea)	9	1	2	2	2	0	3
Edestin	9	0	0	0	0	0	0
Hordein	7	0	0	2	0	0	5
Egg white, dry	3	0	0	0	1	0	2
Prolamin (oat)	2	0	0	0	1	0	1
Globulin (castor bean)	1	0	1	0	0	0	0
Cocoanut protein	1	0	0	0	0	0	1

C. WITH GLIADIN FROM WHEAT AS ANTIGEN

Rabbit 1.—Injected with a total of 1.6 gm. gliadin from wheat from Oct. 19 to Oct. 22, 1915. Blood was drawn and serum obtained Oct. 26. The rabbit died shortly afterward as the result of wound infection.

TABLE 12

RESULTS OF ABDERHALDEN TEST EMPLOYING GLIADIN (WHEAT) AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, I	Oct. 26	24	0.01 gliadin (wheat)	1	+
			0.01 gliadin (rye)	1	+ Strong
			0.1 edestin	1	+
			0.1 prolamin (oat)	1	0
				1*	Faint trace

* Control.

Rabbit 2.—Injected with a total of 5.15 gm. gliadin from wheat from Oct. 30, 1915, to March 3, 1916. Blood was drawn and serum obtained Nov. 28, Dec. 8, 1915, Jan. 21, Feb. 17, 25, and March 6, 1916. Part of the serum obtained Jan. 21, as well as that of Feb. 17, 25, and March 2, was predialyzed to running salt solution (0.85%) for about 6 hours.

TABLE 13

RESULTS OF ABDERHALDEN TEST EMPLOYING GLIADIN (WHEAT) AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, II	Nov. 28	18	0.1 gliadin (wheat)	1	++
			0.1 gliadin (rye)	1	0
			0.1 legumin (pea)	1	+
			0.1 edestin	1	0
			0.1 hordein	1	Trace (?)
			0.1 globulin (squash seed)	1	+
			0.1 prolamin (oat)	1	Strong trace
				1*	0
2, III	Dec. 8	18	0.1 gliadin (wheat)	1	+
			0.1 gliadin (wheat)	1.5	++
				1.5*	Trace
			0.1 gliadin (rye)	1	0
			0.1 legumin (pea)	1	+
			0.1 edestin	1	0
			0.1 hordein	1	0
			0.1 globulin (squash seed)	1	+
			0.1 prolamin (oat)	1	Trace (?)
			0.1 alcohol-sol. protein from millet	1	0
				1*	0
3, IV	Jan. 21	16	0.1 gliadin (wheat)	1	+
			0.1 legumin (pea)	1	Strong trace
			0.1 edestin	1	+
			0.1 hordein	1	Strong trace
			0.1 globulin (squash seed)	1	Strong trace
				1*	0
4, V†	Jan. 21	17	0.1 gliadin (wheat)	1	+
			0.1 legumin (pea)	1	0
			0.1 edestin	1	Faint trace
			0.1 hordein	1	Faint trace
			0.1 globulin (squash seed)	1	Faint trace
				1*	0
5, VI	Feb. 17	17	0.1 gliadin (wheat)	1	+
			0.2 gliadin (wheat)	1.5	++
				1.5*	0
			0.1 gliadin (rye)	1	+
			0.1 legumin (pea)	1	Strong trace
			0.1 edestin	1	Strong trace
			0.1 hordein	1	Faint trace
			0.1 globulin (squash seed)	1	Strong trace
			0.1 prolamin (oat)	1	Strong trace
			0.1 egg white, dry	1	Faint trace
				1*	0
6, VII	Feb. 25	18	0.1 gliadin (wheat)	1	+
			0.2 gliadin (wheat)	1.5	+
				1.5*	0
			0.1 gliadin (rye)	1	0
			0.1 legumin (pea)	1	Faint trace
			0.1 edestin	1	0
			0.1 hordein	1	0
			0.1 globulin (squash seed)	1	0
			0.1 prolamin (oat)	1	0
			0.1 egg white, dry	1	++ ‡
				1	0

* Control.

† The serum in Experiment V was of the same sample as that in Experiment IV, but it was predialyzed before use. The reactions are weaker than in the latter experiment, but they nevertheless correspond fairly well.

‡ This inexplicable reaction is probably the result of faulty technic.

TABLE 13—*Continued*

RESULTS OF ABDERHALDEN TEST EMPLOYING GLIADIN (WHEAT) AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
7, VIII	Mar. 6	18	0.1 gliadin (wheat)	1	+
			0.2 gliadin (wheat)	1.5	++
			0.1 gliadin (rye)	1	+
			0.1 legumin (pea)	1	0
			0.1 edestin	1	0
			0.1 hordein	1	0
			0.1 globulin (squash seed)	1	0
			0.1 prolamin (oat)	1	+
			0.1 egg white, dry	1	0
				1*	Trace

* Control.

Rabbit 3.—Injected with a total of 1.75 gm. of gliadin from wheat from Jan. 13 to March 3, 1916. Blood was drawn and serum obtained on Feb. 14, 25, and March 6.

TABLE 14

RESULTS OF ABDERHALDEN TEST EMPLOYING GLIADIN (WHEAT) AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, IX	Feb. 17	18	0.1 gliadin (wheat)	1	+
			0.2 gliadin (wheat)	1.5	++
				1.5*	Faint trace
			0.1 gliadin (rye)	1	+
			0.1 legumin (pea)	1	Strong trace
			0.1 edestin	1	Faint trace
			0.1 hordein	1	0
			0.1 globulin (squash seed)	1	0
			0.1 egg white, dry	1	0
				1*	Error
2, X	Feb. 25	18	0.1 gliadin (wheat)	1	++
			0.2 gliadin (wheat)	1.5	++
				1.5*	Faint trace
			0 1 gliadin (rye)	1	+
			0.1 legumin (pea)	1	Faint trace
			0.1 edestin	1	Faint trace
			0.1 hordein	1	0
			0.1 globulin (squash seed)	1	0
			0.1 prolamin (oat)	0.75	0
			0.1 egg white, dry	1	0
				1*	0
3, XI	Mar. 6	18	0.1 gliadin (wheat)	1	+
			0.1 gliadin (rye)	1	+
			0.1 legumin (pea)	1	0
			0.1 edestin	1	Trace (?)
			0.1 hordein	1	0
			0.1 globulin (squash seed)	1	0
			0.1 prolamin (oat)	1	0
				1*	0

* Control.

A summary of the experiments with gliadin (wheat) is given in Table 15.

TABLE 15

Protein	Number of Tests	"++ Strong" or "++"	"+ Strong" or "+"	Strong Trace	Trace or Faint Trace	Question-able	No Reaction
Gliadin (wheat)	16	6	10	0	0	0	0
Gliadin (rye)	9	0	6	0	0	0	3
Legumin (pea)	10	0	2	3	2	0	3
Edestin	11	0	2	1	2	2	4
Hordein	10	0	0	1	2	1	6
Globulin (squash seed)	10	0	2	2	1	0	5
Prolamin (oat)	8	0	2	2	0	1‡	3
Egg white, dry	5	0	0	0	1	1	3
Alcohol-soluble protein from millet	1	0	0	0	0	0	1

‡ See note (†) Table 13.

D. With Hordein as Antigen

Rabbit 1.—Injected with a total of 1.6 gm. of hordein from Jan. 18 to March 13, 1916. Blood was drawn and serum obtained Feb. 14, 29, and March 17. All the sera were predialyzed to running salt solution (0.85%) for 7, 7½, and 7½ hours respectively.

TABLE 16

Results of Abderhalden Test Employing Hordein as Antigen

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, I	Feb. 14	19	0.1 hordein	1	+
			0.2 hordein	1.5	0
				1.5*	0
			0.1 globulin (squash seed)	1	0
			0.1 gliadin (wheat)	1	+
			0.1 gliadin (rye)	1	0
			0.1 edestin	1	0
			0.1 legumin (pea)	1	0
			0.1 prolamin (oat)	1	+
			0.1 egg white, dry	1	+
				1*	+ (?)†
2, II	Feb. 29	18	0.1 hordein	1	+
			0.2 hordein	1.5	+
				1.5*	0
			0.1 globulin (squash seed)	1	+
			0.1 gliadin (wheat)	1	+
			0.1 gliadin (rye)	1	+
			0.1 edestin	1	+
			0.1 legumin (pea)	1	Faint trace
			0.1 prolamin (oat)	1	+
			0.1 egg white, dry	1	+
				1*	0
3, III	Mar. 17	20	0.1 hordein	1	+
			0.1 hordein	1	+
			0.1 globulin (squash seed)	1	+
			0.1 gliadin (wheat)	1	Strong trace
			0.1 gliadin (rye)	1	0
			0.1 edestin	1	Trace (?)
			0.1 legumin (pea)	1	0
				1*	0

* Control.
† Probably an error, since 1.5 c.c. serum (as control) in the same experiment was negative.

Rabbit 2.—Injected with a total of 1.5 gm. hordein from Jan. 18 to March 17, 1916. Blood was drawn and serum obtained Feb. 14, 29, and March 17. None of the sera was predialyzed. The reaction of the experiment of March 17 was questionable and is discarded. The serum apparently contained too many dialyzable substances which in themselves gave a strong reaction.

TABLE 17

RESULTS OF ABDERHALDEN TEST EMPLOYING HORDEIN AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, IV	Feb. 4	17	0.1 hordein	1	+
			0.2 hordein	1.5	+
				1.5*	0
			0.1 globulin (squash seed)	1	Trace
			0.1 gliadin (wheat)	1	Strong trace
			0.1 gliadin (rye)	1	++
			0.1 edestin	1	+
			0.1 legumin (pea)	1	Strong trace
				1*	0
2, V	Feb. 29	18	0.1 hordein	1	++
			0.2 hordein	1.5	0
				1.5*	0
			0.1 globulin (squash seed)	1	++
			0.1 gliadin (wheat)	1	0
			0.1 edestin	1	0
			0.1 legumin (pea)	1	Faint (?)
			0.1 prolamin (oat)	1	0
			0.1 egg white, dry	1	0
				1*	0

* Control.

A summary of the experiments with hordein is given in Table 18.

TABLE 18

SUMMARY OF RESULTS OF ABDERHALDEN TESTS EMPLOYING HORDEIN AS ANTIGEN

Protein	Number of Tests	"++ Strong" or "++"	"+ Strong" or "+"	Strong Trace	Trace or Faint Trace	Questionable	No Reactions
Hordein	10	1	7	0	0	0	2
Globulin (squash seed)	5	1	2	0	1	0	1
Gliadin (wheat)	5	0	2	2	0	0	1
Gliadin (rye)	4	1	1	0	0	0	2
Edestin	5	0	2	0	1	0	2
Legumin (pea)	5	0	0	1	1	1	2
Prolamin (oat)	3	0	2	0	0	0	1
Egg white, dry	3	0	2	0	0	0	1

E. WITH PEA LEGUMIN AS ANTIGEN

Rabbit 1.—Injected with a total of 4.5 gm. pea legumin from Oct. 22 to Dec. 6, 1915. Blood was drawn and serum obtained Nov. 10, 27, and Dec. 6. The experiment of Nov. 10 was entirely negative, no reactive power apparently having been developed in the serum.

TABLE 19

RESULTS OF ABDERHALDEN TEST EMPLOYING PEA LEGUMIN AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, I	Nov. 27	18	0.01 legumin (pea)	1	Strong trace
			0.01 edestin	1	Strong trace
			0.01 globulin (squash seed)	1	+
			0.01 gliadin (wheat)	1	++
				1*	Trace
2, II	Dec. 21	16	0.1 legumin (pea)	1	++
			0.1 edestin	1	0
			0.1 hordein	1	0
			0.1 globulin (squash seed)	1	Trace (?)
			0.1 gliadin (wheat)	1	+
			0.1 legumin (horse bean)	1	Strong trace
				1*	0

* Control

Rabbit 2.—Injected with a total of 1.9 gm. pea legumin from Jan. 18 to April 9, 1916. Blood was drawn and serum obtained Feb. 8, March 1, 16, April 9, and 12. The serum of Feb. 8 and 16, and of April 9 and 12, were predialyzed to running salt solution (0.85%) for 6, 6, 7, and 6 hours, respectively, before use.

TABLE 20

RESULTS OF ABDERHALDEN TEST EMPLOYING PEA LEGUMIN AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, III	Feb. 8	18	0.1 legumin (pea)	1	+
			0.5 legumin (pea)	1.5	+
				1.5*	0
			0.1 edestin	1	0
			0.1 hordein	1	+
			0.1 globulin (squash seed)	1	++
			0.1 gliadin (wheat)	1	+
				1*	0
2, IV	Feb. 15	18	0.1 legumin (pea)	1	++
			0.2 legumin (pea)	1.5	++
				1.5*	Strong trace
			0.1 edestin	1	+
			0.1 hordein	1	Faint trace ?
			0.1 globulin (squash seed)	1	+
			0.1 gliadin (wheat)	1	+
			0.1 egg white, dry	1	0
			0.1 glycinin (soy bean)	1	Trace
			0.1 legumin (horse bean)	1	Trace
			0.1 legumin (vetch)	1	Trace
			0.1 vicilin (pea)	1	Trace
				1*	0
3, V	Mar. 1	18	0.1 legumin (pea)	1	+
			0.2 legumin (pea)	1.5	++
				1.5*	Strong trace
			0.1 edestin	1	0
			0.1 hordein	1	0
			0.1 globulin (squash seed)	1	0
			0.1 gliadin (wheat)	1	0
			0.1 prolamin (oat)	1	Trace
			0.1 egg white, dry	1	0
			0.1 legumin (horse bean)	1	Trace
				1*	0
4, VI	Mar. 16	18	0.1 legumin (pea)	1	
			0.1 phaseolin (adzuki bean)	1	++
			0.1 glycinin (soy bean)	1	Trace
			0.1 legumin (horse bean)	1	+
			0.1 legumin (vetch)	1	++
			0.1 vicilin (pea)	1	+
			0.1 legumin (lentil)	1	+
			0.1 vignin (cow pea)	1	++
				1*	0
5, VII	April 12	18	0.1 legumin (pea)	1	+
			0.1 phaseolin (adzuki bean)	1	Faint trace
			0.1 glycinin (soy bean)	1	0
			0.1 legumin (vetch)	1	0
			0.1 vicilin (pea)	1	Faint trace
			0.1 legumin (lentil)	1	Faint trace
				1*	Faint trace
6, VIII	April 12	18	0.1 legumin (pea)	1	++
			0.1 phaseolin (adzuki bean)	1	0
			0.1 glycinin (soy bean)	1	0
			0.1 legumin (vetch)	1	0
			0.1 vicilin (pea)	1	+
			0.1 legumin (lentil)	1	0
			0.1 vignin (cow pea)	1	0
				1*	0

* Control.

Rabbit 3.—Injected with a total of 1.9 gm. pea legumin from Jan. 10 to April 9, 1916. Blood was drawn and serum obtained Feb. 8, 15, March 1, 16, April 9, and 12. The sera of March 1, March 16, April 9, and April 12 were predialyzed to running salt solution (0.85%) for 5, 8, 7, and 6 hours, respectively. The experiment of April 12 was questionable and is discarded for reasons previously indicated.

TABLE 21

RESULTS OF ABDERHALDEN TEST EMPLOYING PEA LEGUMIN AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, IX	Feb. 8	18	0.1 legumin (pea)	1	+
			0.1 edestin	1	+ Strong
			0.1 hordein	1	0
			0.1 globulin (squash seed)	1	0
			0.1 gliadin (wheat)	1	0
			0.1 glycinin (soy bean)	1	0
			0.1 legumin (horse bean)	1	0
			0.1 legumin (vetch)	1	0
			0.1 vicilin (pea)	1	0
			0.1 legumin (lentil)	1	0
				1*	0
2, X	Feb. 15	17	0.1 legumin (pea)	1	++
			0.2 legumin (pea)	1.5	++
				1.5*	0
			0.1 edestin	1	0
			0.1 hordein	1	Strong trace
			0.1 globulin (squash seed)	1	0
			0.1 gliadin (wheat)	1	Strong trace
			0.1 egg white, dry	1	Trace
			0.1 glycinin (soy bean)	1	Strong trace
			0.1 legumin (horse bean)	1	Strong trace
			0.1 legumin (vetch)	1	Strong trace
			0.1 vicilin (pea)	1*	0
3, XI	Mar. 1	18	0.1 legumin (pea)	1	Trace
			0.2 legumin (pea)	1.5	++
				1.5*	0
			0.1 edestin	1	0
			0.1 hordein	1	0
			0.1 globulin (squash seed)	1	0
			0.1 gliadin (wheat)	1	++
			0.1 prolamin (oat)	1	0
			0.1 egg white, dry	1	0
				1*	0
4, XII	Mar. 16	18	0.1 legumin (pea)	1	+
			0.1 phaseolin (adzuki bean)	1	Trace (?)
			0.1 glycinin (soy bean)	1	Trace (?)
			0.1 legumin (horse bean)	1	+
			0.1 legumin (vetch)	1	Trace (?)
			0.1 vicilin (pea)	1	+
			0.1 legumin (lentil)	1	+
			0.1 vignin (cow pea)	1	+
			0.1 globulin (castor bean)	1	++
				1*	0
5, XIII	April 9	19	0.1 legumin (pea)	1	+
			0.1 phaseolin (adzuki bean)	1	Trace
			0.1 glycinin (soy bean)	1	Trace
			0.1 legumin (vetch)	1	Trace
			0.1 vicilin (pea)	1	++
			0.1 legumin (lentil)	1	Trace
			0.1 vignin (cow pea)	1	Trace
				1*	0

* Control

A summary of the experiments with pea legumin is given in Table 22.

TABLE 22

SUMMARY OF RESULTS OF ABDERHALDEN TESTS EMPLOYING PEA LEGUMIN AS ANTIGEN

Protein	Number of Tests	"++ Strong" or "++"	"+ Strong" or "+"	Strong Trace	Trace or Faint Trace	Question-able	No Reaction
Legumin (pea)	18	8	8	1	1	0	0
Edestin	8	0	2	1	0	0	5
Hordein	7	0	1	1	0	1	4
Globulin (squash seed)	8	1	2	0	0	1	4
Gliadin (wheat)	8	2	3	1	0	0	2
Prolamin (oat)	2	0	0	0	1	0	1
Egg white, dry	3	0	0	0	1	0	2
Phaseolin (adzuki bean)	5	1	0	0	2	1	1
Glycinin (soy bean)	8	0	0	1	3	1	3
Legumin (horse bean)	6	0	2	2	1	0	1
Legumin (vetch)	8	1	0	1	2	1	3
Vicilin (pea)	8	1	3	1	2	0	1
Legumin (lentil)	6	0	2	0	2	0	2
Vignin (cow pea)	4	1	1	0	1	0	1
Globulin (castor bean)	1	1	0	0	0	0	0

F. WITH PHASEOLIN FROM ADZUKI BEAN AS ANTIGEN

Rabbit 1.—Injected with a total of 0.7 gm. phaseolin from March 15 to April 8, 1916. Blood was drawn and serum obtained March 29, April 8, and 11. The sera were all predialyzed to running salt solution (0.85%) for 8, 10, and 6 hours, respectively.

TABLE 23

RESULTS OF ABDERHALDEN TESTS EMPLOYING PHASEOLIN (ADZUKI BEAN) AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, I	Mar. 29	22	0.1 phaseolin	1	+
			0.1 glycinin (soy bean)	1	0
			0.1 legumin (pea)	1	0
			0.1 legumin (vetch)	1	Strong trace
			0.1 vicilin (pea)	1	++
			0.1 legumin (lentil)	1	Trace
			0.1 vignin (cow pea)	1	Trace
				1*	0
2, II	April 8	19	0.1 phaseolin	1	+
			0.1 glycinin (soy bean)	1	0
			0.1 legumin (pea)	1	0
			0.1 legumin (vetch)	1	Trace
			0.1 vicilin (pea)	1	Trace
			0.1 legumin (lentil)	1	0
			0.1 vignin (cow pea)	1	0
				1*	0
3, III	April 11	20	0.1 phaseolin	1	Strong trace
			0.1 glycinin (soy bean)	1	Strong trace
			0.1 legumin (pea)	1	Strong trace
			0.1 legumin (vetch)	1	+
			0.1 vicilin (pea)	1	+
			0.1 legumin (lentil)	1	Trace
			0.1 vignin (cow pea)	1	+
				1*	Trace

* Control.

Rabbit 2.—Injected with a total of 0.7 gm. phaseolin from March 15 to April 8, 1916. Blood was drawn and serum obtained March 29, April 8, and 11. The sera were all predialyzed to running salt solution (0.85%) for 8, 10, and 6 hours, respectively.

TABLE 24

RESULTS OF ABDERHALDEN TESTS EMPLOYING PHASEOLIN (ADZUKI BEAN) AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, IV	Mar. 29	22	0.1 phaseolin	1	Trace
			0.1 glycinin (soy bean)	1	Trace
			0.1 legumin (pea)	1	+
			0.1 legumin (vetch)	1	Trace
			0.1 vicilin (pea)	1	Trace
			0.1 legumin (lentil)	1	+
			0.1 vignin (cow pea)	1*	0
2, V	April 9	19	0.1 phaseolin	1	+ Strong
			0.1 glycinin (soy bean)	1	Faint trace
			0.1 legumin (pea)	1	Faint trace
			0.1 legumin (vetch)	1	Faint trace
			0.1 vicilin (pea)	1	Faint trace
			0.1 legumin (lentil)	1	Faint trace
				1*	0
3, VI	April 11	20	0.1 phaseolin	1	Strong trace
			0.1 glycinin (soy bean)	1	Strong trace
			0.1 legumin (pea)	1	Strong trace
			0.1 legumin (vetch)	1	+
			0.1 vicilin (pea)	1	+
			0.1 legumin (lentil)	1	Faint trace
			0.1 vignin (cow pea)	1	Faint trace
				1*	0

* Control.

A summary of the experiments with phaseolin is given in Table 25.

TABLE 25

SUMMARY OF RESULTS OF ABDERHALDEN TESTS EMPLOYING PHASEOLIN (ADZUKI BEAN) AS ANTIGEN

Protein	Number of Tests	"++ Strong" or "++"	"+ Strong" or "+"	Strong Trace	Trace or Faint Trace	Questionable	No Reactions
Phaseolin	6	0	3	2	1	0	0
Glycinin	6	0	0	2	2	0	2
Legumin (pea)	6	0	1	2	1	0	2
Legumin (vetch)	6	0	2	1	3	0	0
Vicilin (pea)	6	1	2	0	3	0	0
Legumin (lentil)	6	0	1	0	3	0	2
Vignin (cow pea)	5	0	1	0	3	0	1

G. WITH GLYCININ FROM THE SOY BEAN AS ANTIGEN

Rabbit 1.—Injected with a total of 0.7 gm. glycinin from March 15 to April 10, 1916. Blood was drawn and serum obtained April 7, 10, and 13. The sera were all predialyzed to running salt solution (0.85%) for 7, 6 and 7 hours, respectively. The experiment of April 13 was questionable and is discarded for reasons already indicated.

TABLE 26

RESULTS OF ABDERHALDEN TESTS EMPLOYING GLYCININ (SOY BEAN) AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, I	April 7	22	0.1 glycinin (soy bean)	1	+
			0.1 phaseolin (adzuki bean)	1	Trace
			0.1 legumin (pea)	1	Trace
			0.1 legumin (vetch)	1	++
			0.1 vicilin (pea)	1	+
			0.1 legumin (lentil)	1	+
			0.1 vignin (cow pea)	1	+
				1*	0
2, II	April 10	19	0.1 glycinin (soy bean)	1	+
			0.1 phaseolin (adzuki bean)	1	+
			0.1 legumin (pea)	1	Trace
			0.1 legumin (vetch)	1	Trace
			0.1 vicilin (pea)	1	++
			0.1 legumin (lentil)	1	+
			0.1 vignin (cow pea)	1	+
				1*	0

* Control.

Rabbit 2.—Injected with a total of 0.7 gm. glycinin from March 15 to April 10, 1916. Blood was drawn and serum obtained April 7, 10, and 13. The sera were all predialyzed to running salt solution (0.85%) for 7, 6, and 7 hours, respectively.

TABLE 27

RESULTS OF ABDERHALDEN TESTS EMPLOYING GLYCININ (SOY BEAN) AS ANTIGEN

Test	Date	Incubation, hr.	Substrate, gm.	Serum, c.c.	Reaction
1, III	April 7	22	0.1 glycinin (soy bean)	1	Trace
			0.1 phaseolin (adzuki bean)	1	Trace
			0.1 legumin (pea)	1	Trace
			0.1 legumin (vetch)	1	Trace
			0.1 vicilin (pea)	1	Trace
			0.1 legumin (lentil)	1	Trace
			0.1 vignin (cow pea)	1	+
				1*	Trace ?
2, IV	April 10	19	0.1 glycinin (soy bean)	1	+
			0.1 phaseolin (adzuki bean)	1	+
			0.1 legumin (pea)	1	Trace
			0.1 legumin (vetch)	1	0
			0.1 vicilin (pea)	1	Trace
			0.1 legumin (lentil)	1	+
			0.1 vignin (cow pea)	1	+
				1*	0
3, V	April 13	19	0.1 glycinin (soy bean)	1	+
			0.1 phaseolin (adzuki bean)	1	+ Strong
			0.1 legumin (pea)	1	Strong trace
			0.1 legumin (vetch)	1	Trace
			0.1 vicilin (pea)	1	Trace
			0.1 legumin (lentil)	1	Trace
			0.1 vignin (cow pea)	1	+
				1*	0

* Control.

A summary of the experiments with glycinin is given in Table 28.

TABLE 28

SUMMARY OF RESULTS OF ABDERHALDEN TESTS EMPLOYING GLYCININ (SOY BEAN) AS ANTIGEN

Protein	Number of Tests	"++ Strong" or "++"	"+ Strong" or "+"	Strong Trace	Trace or Faint Trace	Question-able	No Reaction
Glycinin (soy bean)	5	0	4	0	1	0	0
Phaseolin (adzuki bean)	5	0	3	0	2	0	0
Legumin (pea)	5	0	0	1	4	0	0
Legumin (vetch)	5	1	0	0	3	0	1
Vicilin (pea)	5	1	1	0	3	0	0
Legumin (lentil)	5	0	3	0	2	0	0
Vignin (cow pea)	5	0	5	0	0	0	0

H. WITH EGG WHITE AS ANTIGEN

In these experiments rabbits were injected intravenously with a 33⅓% solution of fresh filtered egg white in an 0.85% NaCl solution. The amounts introduced varied from 1.5 c.c. to 10 c.c., and the injections were often repeated as frequently as each day. The experiments were in a sense to serve as controls on those in which vegetable proteins were employed, not because of any close relationship between egg white and these proteins, but to determine whether some interaction might take place between proteins so highly dissimilar. It will have been noted already that the sera of some of the animals immunized to the vegetable proteins seemed to interact with egg white.

Rabbit 1.—Injected with a total of 47 c.c. of a 33⅓% solution of egg white Nov. 5 to 29, 1915. Blood was drawn and serum obtained Nov. 11, 17, 23, and 29. That obtained Nov. 11 gave no reaction with any of the substances employed.

TABLE 29

RESULTS OF ABDERHALDEN TEST EMPLOYING EGG WHITE AS ANTIGEN

Test	Date	Incubation, hr.	Substrate	Serum, c.c.	Reaction
1, I	Nov. 17	20	0.5 gm. coagulated egg white	1	++
			1 c.c. egg white (conc.)	1	++
				1*	Trace
2, II	Nov. 23	19	1 c.c. 5% egg white	1	0
			1 c.c. egg white (conc.)	1	+
			0.01 gm. globulin (squash seed)	1	0
				1*	Trace
3, III	Nov. 29	19	1 c.c. egg white (conc.)	1	++
			0.1 gm. legumin (pea)	1	+
			0.1 gm. gliadin (wheat)	1	++
			0.1 gm. edestin	1	0
				1*	0

* Control.

Rabbit 2.—Injected with a total of 24 c.c. of a 33⅓% solution of egg white from Jan. 13 to Feb. 4, 1916. Blood was drawn and serum obtained Feb. 7.

TABLE 30

RESULTS OF ABDERHALDEN TEST EMPLOYING EGG WHITE AS ANTIGEN

Test	Date	Incubation, hr.	Substrate	Serum, c.c.	Reaction
1, IV	Feb. 7	17	1 c.c. egg white (conc.)	1.7	Strong trace
			1 c.c. egg white (conc.)	1	Strong trace
			1 c.c. 5% egg white	1	0
			1 c.c. 5% egg white	1.5	+
				1.5*	0
			0.1 gm. legumin (pea)	1	+ Strong
			0.1 gm. gliadin (wheat)	1	0
			0.1 gm. gliadin (rye)	1	Faint trace
			0.1 gm. edestin	1	0
			0.2 gm. edestin	1	0
			0.1 gm. globulin (squash seed)	1	++
			0.1 gm. hordein	1	0
			0.1 gm. prolamin (oat)	1	Faint trace

* Control.

A summary of the experiments with egg white is given in Table 31.

TABLE 31

SUMMARY OF RESULTS OF ABDERHALDEN TESTS EMPLOYING EGG WHITE AS ANTIGEN

Protein	Number of Tests	"++ Strong" or "++"	"+ Strong" or "+"	Strong Trace	Trace or Faint Trace	Questionable	No Reaction
Egg white (conc.)	5	2	1	2	0	0	0
Egg white (5%)	3	0	1	0	0	0	2
Egg white (coagulated; 0.5 gm.)	1	1	0	0	0	0	0
Legumin (pea)	2	0	2	0	0	0	0
Gliadin (wheat)	2	1	0	0	0	0	1
Gliadin (rye)	11	0	0	0	1	0	0
Edestin	3	0	0	0	0	0	3
Globulin (squash seed)	2	1	0	0	0	0	1
Hordein	1	0	0	0	0	0	1
Prolamin (oat)	1	0	0	0	1	0	0

GENERAL DISCUSSION

As can be seen from the tables the purified vegetable proteins can be utilized in immunizing animals for the Abderhalden reaction. Sera are thus obtained which when added to various vegetable proteins cause an interaction of some kind, whereby dialyzable products can be detected. The results of these interactions from the individual experiments (Tables 7, 11, 15, 18, 20, 25, 28, and 31) are placed in Table 32 in the form of a general summary.

In analyzing these tables certain facts should be borne in mind. As has been stated, it was not possible to obtain an equal number of reactions for all the proteins employed, altho this would have been highly desirable. Hence it may appear (Table 32) that whereas 16 of 18, or 88% of the tests made with edestin immune serum against edestin were positive, 2 tests, or 100%, using rye gliadin were positive. Obviously conclusions drawn on such a basis would be fallacious. It should be pointed out also that Table 32 includes all the reactions in the various experiments which were read "positive" ranging from "strong trace" to "++ strong." The weaker reactions have been eliminated, for it is here that the greatest uncertainty lies in making the readings.

The performance of a single experiment is beset by many pitfalls, which can be eliminated only after long and diligent labor. The quantitative reading of the color reactions is much governed by subjective influences. We avoided this at times by asking another person to make the readings. Faulty dialyzing tubes no doubt play a serious part as many investigators have emphasized. Indeed, any one who has worked extensively with the dialysis method of Abderhalden is familiar with the laborious care which must be exercised continually and the uncertainty which may attend the most careful efforts. One hesitates to use a method which does not permit of clear cut and definite results.

It is to be noted, however, that the reactions between immune serum and homologous antigen tend to be specific. That is, when a sufficient number of tests have been made to warrant comparison, a higher percentage of the reactions are positive when the specific substrate is employed than when a nonspecific substrate is employed. For example (quoting from Table 32), 16 of 18 tests were positive when edestin immune serum was used against edestin, while 4 of 7 were positive when wheat gliadin was used as substrate. Again, wheat gliadin immune serum reacted 16 times out of 16 with gliadin as substrate, and 3 times out of 11 with edestin as substrate. The individual summaries will indicate that the greater number of the strongest reactions tended to occur with the specific protein, while the greatest number of weaker reactions occurred with other substrates. The totals in Table 32 indicate several interesting features. Thus edestin and hordein and egg white, when tested as substrates against heterologous sera, yield a low percentage of positive results as compared with negative results. Squash-seed globulin, excelsin, gliadin from rye, oat prolamin, pea

TABLE 32

GENERAL SUMMARY OF RESULTS OF ABDERHALDEN TESTS EMPLOYING VEGETABLE PROTEINS AS ANTIGEN

Proteins Employed as Substrates	Table References	Substrates																											
		Edestin			Globulin (Squash Seed)			Excelsin			Gliadin (Wheat)			Gliadin (Rye)			Hordein			Prolamin (Oat)			Alcohol-Soluble Protein from Millet			Globulin (Castor Bean)		
		No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative
Edestin.....	7	18	16	1*	6	1	5	7	2	4	7	4	2	2	2	0	7	1	6	2	1	1	1	0	1	1	0	1
Globulin.... (squash seed)	11	9	0	9	15	7	6	10	4	6	9	5	2	5	1	4	7	2	5	2	0	1	.....			1	1	0
Gliadin..... (wheat)	15	11	3	4	10	4	5	.....			16	16	0	9	6	3	10	1	6	8	4	3	1	0	1	.....		
Hordein.....	18	5	2	2	5	3	1	.....			5	4	1	4	2	2	10	8	2	3	2	1	.....			.....		
Legumin.... (pea)	22	8	3	5	8	3	4	.....			8	6	2	.....			7	2	4	2	0	1	.....			1	1	0
Phaseolin...	25	.....			.....			.....			.....			.....			.....			.....			.....			.....		
Glycinin.....	28	.....			.....			.....			.....			.....			.....			.....			.....			.....		
Egg white...	31	3	0	3	2	1	1	.....			2	1	1	1	0	0	1	0	1	1	0	0	.....			.....		
Totals†...	..	36	8	23	31	12	16	17	6	10	31	20	8	21	11	9	32	6	22	18	7	7	2	0	2	3	2	1

* Questionable and trace or faint-trace reactions are not recorded.
† The totals do not include the reactions between an immune serum and its homologous substrate; for example, edestin immune serum as tested against edestin.

legumin, glycinin, legumin from vetch and lentil, and vignin have approximately as many positive as negative reactions when used as substrates against heterologous sera. Gliadin from wheat, phaseolin, and vicilin, however, have a large percentage of positive reactions as compared with negative reactions.

The experiments have moreover shown that a rabbit may react in an absolutely specific manner at one time (Experiment V, Table 2), altho it has not done so in a previous test and does not do so in the serum obtained subsequently. The individual rabbits also appear to differ in their reacting powers. Lake,[2] working out the immunologic relationship of certain of the vegetable proteins as shown by complement-fixation, passive anaphylaxis, and precipitin reactions, states that "anti-sera to the same protein obtained from different individual animals differ in their relations, for some unknown cause;" also, "anti-serum at one stage of its development may be apparently of sharply limited specificity, etc." He indicates that increased antibody content in the serum will cause the latter to react more generally. On the other hand, Pearce and Williams,[7] using kidney tissue as antigen, con-

TABLE 32—*Continued*

GENERAL SUMMARY OF RESULTS OF ABDERHALDEN TESTS EMPLOYING VEGETABLE PROTEINS AS ANTIGEN

Substrates

Cocoanut Protein			Globulin (Black Walnut)			Legumin (Horse Bean)			Legumin (Pea)			Phaseolin			Glycinin			Legumin (Vetch)			Vicilin (Pea)			Legumin (Lentil)			Vignin (Cowpea)			Egg White		
No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative	No. of Tests	Positive	Negative
1	1	0	1	0	1	1	0	1	7	2	4	....			....			....			....			....			....			6	0	6
1	0	1	....			....			9	4	3	....			....			....			....			....			....			3	0	2
....			....			....			10	5	3	....			....			....			....			....			....			5	0	3
....			....			....			5	1	2	....			....			....			....			....			....			3	2	1
....			....			6	4	1	18	17	0	5	1	1	8	1	3	8	2	3	8	5	1	6	2	2	4	2	1	3	0	2
....			....			....			6	3	2	6	5	0	6	2	2	6	3	0	6	3	0	6	1	2	5	1	1			
....			....			....			5	1	0	5	3	0	5	4	0	5	1	1	5	2	0	5	3	0	5	5	0			
....			....			....			2	2	0	....			....			....			....			....			....			9	7	2
2	1	1	1	0	1	7	4	2	44	18	14	10	4	1	14	3	5	19	6	4	19	10	1	17	6	4	14	3	2	20	2	16

clude that "multiple injections tend to a slight selective action." Our results at least indicate that continued injection did not increase the specificity of the serum.

The results obtained by means of the Abderhalden reaction have been so irregular and uncertain that a detailed comparison of the results obtained by this means with the observations on the same proteins made in respect to anaphylaxis and other reactions by Wells, Osborne, and Lake,[2] seems scarcely profitable at this time. I have made a careful comparison of the data published by these observers with those reported in this paper, and have found that while in many instances there is a reasonable agreement, in others there are great and unaccountable discrepancies.

SUMMARY OF EXPERIMENTS

The action of immune sera on purified vegetable proteins as determined by the Abderhalden reaction seems to be only quantitatively specific. Immune sera tend to react more often and more strongly with their specific substrates than with any other substrate. However, the reaction is far from being absolutely specific.

An immune serum may or may not react specifically with its own antigen; in the same test it may not react at all with its antigen, while reacting with other proteins.

Immune sera against closely allied proteins tend to interact with these various antigens. This is especially demonstrated by the interreactions among the proteins from the leguminous seeds.

A greater degree of specificity exists between animal proteins (egg white) and vegetable proteins than between different vegetable proteins.

The serum of an immune rabbit is not constant in its reactive power over a continued period, nor in the degree of specificity it exhibits. There also exists an apparent difference between the sera of the individual rabbits in these regards.

GENERAL CONCLUSIONS

It is demonstrated in this work that the specificity of the Abderhalden reaction (dialysis method) in experimental animals (rabbits) immunized with pure isolated vegetable proteins is far from being absolute. The conditions under which this experimental work was performed were rigidly controlled and the requisite care was constantly exercised in performing all the Abderhalden tests. The biologic interrelationship and specificity of the preparations of vegetable proteins used for this work have been previously tested by means of anaphylaxis, complement-fixation, and precipitin reactions.

Under the conditions of the experiments the Abderhalden reaction is at best only quantitatively specific and even this quantitative specificity is not always exhibited. This is demonstrated by the following observations: (1) an homologous substrate may react specifically with its immune serum, no other protein reacting; (2) it may vary quantitatively in the degree of interreaction with its own immune serum; (3) it may react with its immune serum but no more strongly than do the heterologous substrates tried against the same immune serum; (4) it may give no reaction against its immune serum, while other heterologous proteins may react strongly against this serum; (5) it may react at times more strongly with a heterologous immune serum than with its own immune serum. However, there is an obvious tendency for a substrate to react more often and yield stronger reactions when tested against its homologous immune serum, than when tested against a heterologous immune serum.

When the test is made with closely allied pure vegetable proteins, the Abderhalden reaction tends to be less quantitatively specific the more similar these same proteins are. However, there is no absolute specificity even between widely diverse proteins, such as egg white and pure vegetable protein, as tested in our experiments.

In comparing the results obtained by the Abderhalden test with those obtained by anaphylaxis, by Wells, Osborne and Lake, using the same pure vegetable proteins in each case, it was found that the results present certain resemblances, but also often definite differences. In either reaction the more closely the proteins are allied chemically and physically, the less specific the reactions tend to become. On the whole, however, the results obtained by anaphylaxis are much more constant and specific.